FIFTH COMMISSION DIRECTIVE

of 25 March 1974

establishing Community methods of analysis for the official control of feedingstuffs

(74/203/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community;

Having regard to the Council Directive of 20 July 1970 [1], on the introduction of Community methods of sampling and analysis for the official control of feedingstuffs, as last amended by the Act [2] annexed to the Treaty [3] concerning the Accession of new Member States to the European Economic Community and the European Atomic Energy Community, and in particular Article 2 thereof;

Whereas that Directive requires that official controls of feedingstuffs be carried out using Community methods of sampling and analysis for the purpose of checking compliance with requirements arising under the provisions laid down by law, regulation or administrative action concerning the quality and composition of feedingstuffs;

Whereas Directives No 71/250/EEC of 15 June 1971 [4], No 71/393/EEC of 18 November 1971 [5], No 72/199/EEC of 27 April 1972 [6] and No 73/46/EEC of 5 December 1972 [7] have already established a number of Community methods of analysis; whereas the progress of work since then makes it advisable to adopt a fifth set of methods;

Whereas the measures provided for in this Directive are in accordance with the Opinion of the Standing Committee for Feedingstuffs,

HAS ADOPTED THIS DIRECTIVE:

Article 1

The Member States shall require that analyses for official controls of feedingstuffs as regards the content of starch and of starch degradation products of high molecular weight in feedingstuffs containing beet cossettes, beet bulp, dried beet tops or leaves, potato pulp, dried yeasts, products rich in inulin or greaves be carried out according to the method described in Annex I to this Directive.

The general provisions set out in Part 1 (Introduction) of the Annex to the First Commission Directive No 71/250/EEC of 15 June 1971 shall be applicable to the method described in Annex I to this Directive.

Article 2

The Member States shall require that analyses for official controls of feedingstuffs as regard their contents of amprolium, ethopabate, dinitolmide (DOT), nicarbazin and menadione (vitamin K_3) be carried out according to the methods described in Annex II to this Directive.

The general provisions set out in Part 1 (Introduction) of the Annex to the First Commission Directive No 71/250/EEC of 15 June 1971, with the exception of the part dealing with preparation of the sample to be analysed, shall be applicable to the methods described in Annex II to this Directive.

Article 3

The Member States shall, not later than 1 November 1974, bring into force the laws, regulations or administrative provisions necessary to comply with this Directive. They shall forthwith notify the Commission thereof.

Article 4

This Directive is addressed to the Member States.

Done at Brussels, 25 March 1974.

For the Commission

The President

François-Xavier ORTOLI

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[1] OJ No L 170, 3. 8. 1970, p. 2.
[2] OJ No L 73, 27. 3. 1972, p. 14.
[3] OJ No L 73, 27. 3. 1972, p. 5.
[4] OJ No L 155, 12. 7. 1971, p. 13.
[5] OJ No L 279, 20. 12. 1971, p. 7.
[6] OJ No L 123, 29. 5. 1972, p. 6.
[7] OJ No L 83, 30. 3. 1973, p. 21.

ANNEX I

DETERMINATION OF STARCH

— pancreatic method —

1. **Purpose and field of application**

The method makes it possible to determine the content of starch and of starch degradation products of high molecular weight in feedingstuffs containing beet cossettes, beet pulp, dried beet tops or leaves, potato pulp, dried yeasts, products rich in inulin (e.g. cossettes and meal of Jerusalem artichoke) and products containing greaves. The determination should only be carried out when microscopic examination shows that significant quantities of starch are present in the sample.

2. **Principle**

Sugars present in the sample are extracted with ethanol. The starch in the extracted residue is reduced to sugar with pancreatin. The sugars formed are hydrolysed with hydrochloric acid and the glucose formed is determined by the Luff-Schoorl method. The quantity of glucose thus obtained, multiplied by a constant factor, gives the starch content of the sample.

3. **Reagents**

3.1. 90 % (v/v) ethanol, neutral to phenolphthalein.

3.2. Pentan-1-01 (amyl alcohol) a.p.

3.3. Toluene

3.4. Butter solution : dissolve in water 9·078 g potassium dihydrogen phosphate KH_2PO_4 and 11·876 g disodium hydrogen phosphate $Na_2HPO_4\ 2H_2O$. Make up to 1 litre with water.

3.5. Sodium chloride solution 0·2 N.

3.6. Carrez I solution : dissolve in water 21·9 g zinc acetate $Zn(CH_3COO)_2.\ 2H_2O$ and 3 g glacial acetic acid. Make up to 100 ml with water.

3.7. Carrez II solution : dissolve in water 10·6 g potassium ferrocyanide $K_4\ /Fe\ (CN_6)/\ 3H_2O$. Make up to 100 ml with water.

3.8. Hydrochloric acid N.

3.9. Hydrochloric acid a.p., approximately 8N, d : 1·125.

3.10. Sodium hydroxide solution a.p., approximately 10 N, d : 1·33.

3.11. Indicator : 0·1 % (w/v) methylorange solution.

3.12. Pancreatin in powder form, as per the directions in point 8. Keep in stoppered flasks, protected from light and moisture.

3.13. Luff-Schoorl reagent : Pour the citric acid solution (3.13.2) into the sodium carbonate solution (3.13.3) stirring carefully during the addition. Then add the copper sulphate solution (3.13.1) and make up to 1 litre with water. Leave to stand overnight and filter. Check the normality of the reagent thus obtained (Cu 0·1 N ; $Na_2\ CO_3$ 2N). The pH of the solution must be approximately 9·4.

3.13.1. Copper sulphate solution : dissolve 25 g copper sulphate a.p. $Cu\ SO_4.5H_2O$ in 100 ml water.

3.13.2. Citric acid solution : dissolve 50 g citric acid a.p. $C_6H_8O_7.H_2O$ in 50 ml water.

3.13.3. Sodium carbonate solution : dissolve 143·8 g anhydrous sodium carbonate a.p. in approximately 300 ml hot water. Leave to cool.

3.14. Granules of pumice stone purified by boiling in hydrochloric acid, washing in water and drying.

3.15. 30 % (w/v) solution of potassium iodide a.p.

3.16. Sulphuric acid, approximately 6 N, d : 1·18.

3.17. Sodium thiosulphate solution 0·1 N.

3.18. Starch solution : add a mixture of 5 g soluble starch in 30 ml water to 1 litre boiling water. Boil for 3 minutes, leave to cool. This solution should be freshly prepared.

4. Apparatus

4.1. Extractor (see diagram p. 12), consisting of :

4.1.1. A wide-necked 500 ml conical flask ;

4.1.2. A reflux condenser fitted with a bung to the conical flask ;

4.1.3. A sliding spindle in the centre of the reflux condenser, fitted with a hook at its lower end. A peg to hold the spindle firm ;

4.1.4. A metal container for suspending on the spindle hook (4.1.3) and holding the filtration crucible (4.1.5) ;

4.1.5. A filtration crucible for rapid filtration ; max. size of pores : 90 to 150 microns (e.g. porosity one, approximately 30 ml in capacity ;

4.1.6. Filter papers, suitable in shape and size for the filtration crucible.

4.2. Incubator, set at 38 °C.

4.3. 200 ml graduated flasks with a standard ground glass joint and reflux condenser.

4.4. 100 ml graduated flasks with a standard ground glass joint and reflux condenser.

5. Procedure

5.1. *Preparation of the sample*

Crush the sample so that the whole of it will pass through a 0·5 mm sieve. (ISO sieve of R 565 conforms).

5.2. *Extraction*

Weigh to the nearest mg 2 g of the sample and place in the filtration crucible (4.1.5), the bottom of which has first been covered with a filter paper (4.1.6) moistened with ethanol (3.1). Place in the conical flask (4.1.1) 55 ml ethanol (3.1) and a few granules of pumice stone (3.14). Place the filtration crucible in the metal container (4.1.4) and suspend the latter on the spindle hook (4.1.3). Place the reflux condenser over the conical flask and lower the spindle so that the bottom of the crucible just touches the surface of the ethanol. Peg the spindle firmly at this level. Bring the ethanol to boiling point and keep boiling for 3 hours. Then leave to cool and raise the spindle (4.1.3) to bring the crucible as high up as possible in the conical flask. Carefully unstopper the conical flask and allow 45 ml water to flow down along the sides of the flask. Replace the reflux condenser over the Erlenmeyer flask and keep the filtration crucible 10 cm above the surface of the liquid. Bring the liquid to boiling point and keep boiling for 3 hours. Then leave to cool, unstopper the conical flask and withdraw the crucible from the container.

5.3. *Saccharification and hydrolysis*

Place the crucible on a vacuum flask and dry under suction. Transfer the extraction residue to a mortar and grind finely. Using approximately 60 ml water, transfer the powder quantitatively to a 200 ml graduated flask with a standard ground glass joint and add a few drops of amyl alcohol (3.2). Connect a reflux condenser to the flask. Heat to boiling point and keep boiling for 1 hour. Then leave to cool and disconnect the reflux condenser. Add 25 ml buffer solution (3.4), 250 mg pancreatin (3.12), 2·5 ml sodium chloride solution (3.5) and 10 drops toluene (3.3). Shake for 2 minutes, place the flask in the incubator (4.2) and keep there for 21 hours, shaking occasionally. Then leave to cool to room temperature.

Add 5 ml Carrez I solution (3.6) and shake for one minute. Then add 5 ml Carrez II solution (3.7) and shake once again for one minute. Make up to volume with water, mix and filter. Using a pipette, take 50 ml filtrate and place in a 100 ml graduated flask (one may also work on 100 ml filtrate in a 200 ml graduated flask). Add a few drops of indicator (3.11) and acidify with hydrocholoric acid 8 N (3.9) until the indicator turns red. Then add an extra 6·25 ml hydrochloric acid 8 N (3.9) (12·50 ml if working on 100 ml filtrate). Connect the reflux condenser to the flask, bring the solution to boiling point and keep boiling for 1 hour. Leave to cool, neutralize with the sodium hydroxide solution 10 N (3.10) until the indicator turns yellow. Then acidify slightly by adding a little hydrochloric acid N ('3.8), make up to volume with water and mix. Determine the glucose content according to the **Luff-Schoorl method as shown in 5.4.**

5.4 *Titration according to Luff-Schoorl*

Using a pipette, take 25 ml Luff-Schoorl reagent (3.13) and place in a 300 ml conical flask ; add 25 ml, accurately measured, of the solution obtained in 5·3, this should not contain more than 60 mg of glucose. Add two granules of pumice stone (3.14), heat, while shaking manually, over a medium flame and bring the liquid to boiling point in approximately 2 minutes. Immediately place the conical flask on a wire gauze fitted with an asbestos screen which has a hole approximately 6 cm in diameter. Under the wire gauze a flame has first been lit, and this is regulated in such a way that only the bottom of the conical flask is heated. Then connect a reflux condenser to the conical flask. As soon as this is done, boil for 10 minutes exactly. Cool immediately in cold water and after approximately 5 minutes, titrate as follows :

Add 10 ml potassium iodide solution (3.15) and, immediately afterwards, but with care, (because of the risk of extensive foaming) 25 ml sulphuric acid 6 N (3.16). Then titrate with the sodium thiosulphate solution 0·1 N (3.17) until a dull yellow colouring appears, add a few drops of starch indicator (3.18) and complete the titration.

Carry out the same titration on an accurately measured mixture of 25 ml Luff-Schoorl reagent (3.13) and 25 ml water, after adding 10 ml potassium iodide solution (3.15) and 25 ml sulphuric acid 6 N (3.16), without bringing to the boil.

5.5. *Blank test*

Carry out a blank test, applying the procedure described in 5.3 and 5.4, but without a sample.

6. Calculation of results

Using the table in the Annex, determine the quantity of glucose in mg corresponding to the difference between the results of the two titrations (expressed in ml of sodium thiosulphate 0·1 N) and relating both to the sample analysis and the blank test.

The content of starch as a percentage of sample is given by the formula :

$$0{\cdot}72\ (a - b)$$

where :

a = mg of glucose relating to the sample ;
b = mg of glucose relating to the blank test (see observation 7.2).

7. Observations

7.1 Where partially or totally dextrinated starch and lactose are simultaneously present in the sample, the result may be high by 0·5 to 3·0 %. In such cases, the actual starch content is obtained as follows :

(a) Determine the content of reducing sugars in the ethanolic extract obtained in 5.2, and express the result as a percentage of glucose ;

(b) Determine the content of water-soluble reducing sugars in the sample, and express the result as a percentage of glucose ;

(c) Deduct the result obtained in (a) from that obtained in (b) and multiply the difference by 0·9 ;

(d) Deduct the value obtained in (c) from the starch content obtained by applying the method and calculating as shown in 6.

7.2 The quantity of glucose in relation to the blank test is normally 0·25 mg. It may not be greater than 0·50 mg.

8. Directions relating to pancreatin

Physical aspect : yellowish-white, amorphous powder.

Glucose content : the quantity of glucose of the blank test (see 5.5) is normally 0·25 mg. A result greater than 0·50 mg indicates that pancreatin can no longer be used.

Check for iodine consumption : suspend 62·5 mg pancreatin in approximately 50 ml water heated to 25 to 30 °C. Add 1 ml iodine solution 0·1 N. Stir for 2 minutes. Titrate with a solution of sodium thiosulphate 0·1 N in the presence of starch indicator. Consumption of iodine solution by pancreatin must not exceed 0·5 ml.

Check for amylolytic activity : mix 100 ml starch solution (3.18), 5 ml buffer solution (3.4), 0·5 ml sodium chloride solution (3.5) and 62·5 mg pancreatin. Heat the mixture to 25 to 30 °C, stir for 2 minutes. Add 1 ml iodine solution 0·1 N. The blue colouration must have disappeared within 15 minutes exactly of the addition of the iodine solution.

Table of values for 25 ml Luff-Schoorl reagent

ml of $Na_2S_2O_3$ 0·1 N ; 2 minutes heating ; 10 minutes boiling

$Na_2S_2O_3$ 0·1 N	Glucose, fructose, invert sugars $C_6H_{12}O_6$		Lactose $C_{12}H_{22}O_{11}$		Maltose $C_{12}H_{22}O_{11}$		$Na_2S_2O_3$ 0·1 N
ml	mg	difference	mg	difference	mg	difference	ml
1	2·4		3·6		3·9		1
		2·4		3·7		3·9	
2	4·8		7·3		7·8		2
		2·4		3·7		3·9	
3	7·2		11·0		11·7		3
		2·5		3·7		3·9	
4	9·7		14·7		15·6		4
		2·5		3·7		4·0	
5	12·2		18·4		19·6		5
		2·5		3·7		3·9	
6	14·7		22·1		23·5		6
		2·5		3·7		4·0	
7	17·2		25·8		27·5		7
		2·6		3·7		4·0	
8	19·8		29·5		31·5		8
		2·6		3·7		4·0	
9	22·4		33·2		35·5		9
		2·6		3·8		4·0	
10	25·0		37·0		39·5		10
		2·6		3·8		4·0	
11	27·6		40·8		43·5		11
		2·7		3·8		4·0	
12	30·3		44·6		47·5		12
		2·7		3·8		4·1	
13	33·0		48·4		51·6		13
		2·7		3·8		4·1	
14	35·7		52·2		55·7		14
		2·8		3·8		4·1	
15	38·5		56·0		59·8		15
		2·8		3·9		4·1	
16	41·3		59·9		63·9		16
		2·9		3·9		4·1	
17	44·2		63·8		68·0		17
		2·9		3·9		4·2	
18	47·1		67·7		72·2		18
		2·9		4·0		4·3	
19	50·0		71·7		76·5		19
		3·0		4·0		4·4	
20	53·0		75·7		80·9		20
		3·0		4·1		4·5	
21	56·0		79·8		85·4		21
		3·1		4·1		4·6	
22	59·1		83·9		90·0		22
		3·1		4·1		4·6	
23	62·2		88·0		94·6		23